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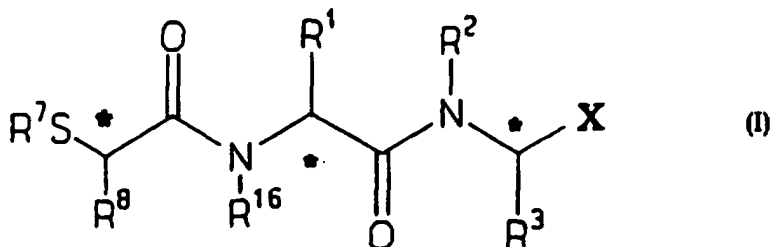
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(54) Title: THIO-SUBSTITUTED PEPTIDES AS INHIBITORS FOR METALLOPROTEINASES AND TNF LIBERATION

(57) Abstract

Peptide compounds of formula (I). Peptides are inhibitors of (matrix) metalloproteinases like collagenase and stromelysin. Furthermore, they inhibit the liberation of tumor necrosis factor α (TNF α).



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**THIO-SUBSTITUTED PEPTIDES AS INHIBITORS FOR
METALLOPROTEINASES AND TNF LIBERATION**

Field of the Invention

This invention relates to a novel class of peptidyl derivatives, to processes for their preparation, and to their use in medicine.

5 **Background to the Invention**

Metalloproteinases, (human fibroblast) collagenase, gelatinase and tumour necrosis factor (TNF) and their modes of action, and also inhibitors thereof and their clinical effects, are disclosed in WO-A-9611209, the content of which is incorporated by reference.

10 In normal tissues, cellular connective tissue synthesis is offset by extracellular matrix degradation, the two opposing effects existing in dynamic equilibrium. Degradation of the matrix is brought about by the action of proteinases released from resident connective tissue cells and invading inflammatory cells, and is due, in part, to the activity of at least four groups of metalloproteinases. These are the collagenases
15 (interstitial collagenase, MMP-1; PMN collagenase, MMP-8, collagenase-3, MMP-13), the gelatinases (gelatinase A, MMP-2, 72kDa-gelatinase, Type IV collagenase; gelatinase B, MMP-9, 92kDa-gelatinase, Type IV collagenase) the stromelysins (proteoglycanase, MMP-3, stromelysin-1, transin; stromelysin-2, MMP-10; stromelysin 3, MMP-11) and the membrane type matrix metalloproteinases (MT-1, MMP-14; MT-2,
20 MMP-15; MT-3, MMP-16 and MT-4, MMP-17). Normally these catabolic enzymes are tightly regulated at the level of their synthesis and secretion and also at the level of their extracellular activity, the latter through the action of specific inhibitors, such as TIMP (tissue inhibitors of metalloproteinase), which form inactive complexes with metalloproteinases, and more general proteinase inhibitors such as α_2 -macroglobulins.

25 The accelerated, uncontrolled breakdown of connective tissues by metalloproteinase catalysed resorption of the extracellular matrix is a feature of many pathological conditions such as rheumatoid arthritis, osteoarthritis, septic arthritis, corneal, epidermal or gastric ulceration; tumour metastasis or invasion; periodontal disease, proteinuria, coronary thrombosis associated with atherosclerotic plaque rupture
30 and bone disease. The inhibitors claimed herein may also be useful in preventing the

pathological squaelae following a traumatic injury that could lead to a permanent disability. These compounds may also have utility as a means for birth control by preventing ovulation or implantation. It can be expected that the pathogenesis of such diseases is likely to be modified in a beneficial manner by the administration of metalloproteinase inhibitors and numerous compounds have been suggested for this purpose [for a general review see R C Wahl, *et al* Ann. Rep, Med. Chem. 25: 175-184, Academic Press Inc., San Diego (1990)].

A number of small peptide like compounds which inhibit metalloproteinases have been described. Perhaps the most notable of these are those relating to angiotensin converting enzyme (ACE) where such agents act to block the conversion of the decapeptide angiotensin I to angiotensin II, a potent pressor substance. Compounds of this type are described in EP-A-0012401. Also, related mercaptoamide peptidyl derivatives have shown ACE inhibitor activity *in vitro* and *in vivo* (H N Weller *et al* (1984), Biochem Biophys. Res. Comm., 125 (1):82-89).

TNF is a cytokine which is produced initially as a cell-associated 28kD precursor. It is released as an active, 17kD form (D-M Jue *et al*, (1990) Biochemistry, 29:8371-8377), which can mediate a large number of deleterious effects *in vivo*. When administered to animals or humans it causes inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase responses, similar to those seen during acute infections and shock states. Chronic administration can also cause cachexia and anorexia. Accumulation of excessive TNF can be lethal.

There is considerable evidence from animal model studies that blocking the effects of TNF with specific antibodies can be beneficial in acute infections, shock states, graft versus host reactions and autoimmune disease. TNF is also an autocrine growth factor for some myelomas and lymphomas and can act to inhibit normal heamatopoiesis in patients with these tumours.

Preventing the production or action of TNF is, therefore, predicted to be a potent therapeutic strategy for many inflammatory, infectious, immunological or malignant diseases. These include, but are not restricted to, septic shock, haemodynamic shock

and sepsis syndrome (Mathison *et al* (1988) *J. Clin. Invest.* 81:1925-1937; Miethke *et al* (1992), *J. Exp. Med.* 175:91-98), post ischaemic reperfusion injury, malaria (Grau *et al* (1989), *Immunol. Rev.* 112:49-70); mycobacterial infection (Barnes *et al* (1992) *Infect. Imm.* 60:1441-6), meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, autoimmune disease, rheumatoid arthritis, multiple sclerosis, radiation damage, toxicity following administration of immunosuppressive monoclonal antibodies such as OKT3 or CAMPATH-1 and hyperoxic alveolar injury.

Current clinical anti-TNF strategies involve the use of corticosteroids such as dexamethasone, and the use of cyclosporin-A or FK506, which are non-specific inhibitors of cytokine gene transcription. Phosphodiesterase inhibitors such as pentoxifylline have been shown to be more specific inhibitors of TNF gene transcription (Endres S. (1991) *Immunol.* 72:56-60, Schandene *et al* (1992), *Immunol.* 76:30-34, Alegre ML, *et al* (1991); *Transplantation* 52:674-679, Bianco *et al* (1991) *Blood* 78:1205-1221). Thalidomide has also been shown to inhibit TNF production by leucocytes (Sampajo *et al* (1991), *J. Exp. Med.* 173:699-703). In experimental settings, anti-TNF monoclonal antibodies, soluble TNF receptors and soluble TNF receptor/immunoadhesins have been shown to specifically inhibit the effects of TNF action (Bagby *et al* (1991) *J. Infect. Dis.* 163:83-88, Charpentier *et al.* (1991) *Presse-med.* 20:2009-2011, Silva *et al* (1990) *J. Infect. Dis.* 162:421-427; Franks *et al* (1991) *Infect. Immun.* 59:2609-2614, Tracey *et al* (1987) *Nature* 330:662-664; Fischer *et al* (1992) *PNAS USA* in press, Lesslauer *et al* (1991) *Eur. J. Immunol.* 21:2883-2886, Ashkenazi *et al* (1991) *PNAS USA* 88:10535-10539).

It has recently been shown that the effects of TNF are mediated by two peptides, TNF α and TNF β . Although these peptides have only 30% homology with each other, they activate the same receptors and are encoded by immediately adjacent genes. As used herein, the term tumour necrosis factor or TNF therefore means tumour necrosis factor α and peptides having a high degrees of sequence homology with, or substantially similar physiological effects to, TNF α , for example TNF β .

One of the objectives of the present invention is to provide compounds which substantially inhibit the release of TNF from cells, and therefore may be used in the treatment of conditions mediated by TNF. Such uses include, but are not limited to, the treatment of inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease.

Compounds which have the property of inhibiting the action of metalloproteinases involved in connective tissue breakdown such as collagenase, stromelysin and gelatinase have been shown to inhibit the release of TNF both *in vitro* and *in vivo* (AJH Gearing *et al* (1994), Nature, 370:555-557; GM McGeehan *et al* (1994), Nature, 370:558-561; WO 93/20047). All of these reported inhibitors contain a hydroxamic acid zinc binding group.

As appreciated by those of skill in the art the significant proportion of homology between human fibroblast collagenase, stromelysin and gelatinase leads to the possibility that a compound that inhibits one enzyme may to some degree inhibit all of them.

Compounds that inhibit collagenase include those encompassed by U.S. Patent No. 4,511,504 issued Apr. 16, 1985 and U.S. Patent No. 4,568,666, issued Feb 4, 1986. Compounds of related structure that are claimed to inhibit stromelysin (proteoglycanase) are encompassed by U.S. Patent No. 4,771,037, issued Sept. 13, 1988.

It is believed that stromelysin and collagenase inhibitors may have utility in preventing articular cartilage damage associated with septic arthritis. Bacterial infections of the joints can elicit an inflammatory response that may then be perpetuated beyond what is needed for removal of the infective agent resulting in permanent damage to structural components. Bacterial agents have been used in animal models to elicit an arthritic response with the appearance of proteolytic activities. See J. P. Case *et al* (1989), J. Clin. Invest., 84:1731-40; R. J. Williams *et al* (1990), Arth. Rheum. 33: 533-41.

It is believed that inhibitors of stromelysin, collagenase and gelatinase may be useful to control tumour metastasis, optionally in combination with current

chemotherapy and/or radiation. See L. M. Matrisian *et al* (1986), *Proc. Natl. Acad. Sci., USA*, 83:9413-7; S. M. Wilhelm *et al* (1987), *Ibid.* 84:6725-29; Werb *et al* (1989), *J. Cell Biol.*, 109:872-889; L. A. Liotta *et al* (1983), *Lab. Invest.*, 49:636-649; Reich *et al* in *Metastasis*, Ciba Foundation Symposium, Wiley, Chicester, 1988, pp. 193-210.

Secreted proteinases such as stromelysin, collagenase and gelatinase play an important role in processes involved in the movement of cells during metastatic tumour invasion. Indeed, there is also evidence that the matrix metalloproteinases are overexpressed in certain metastatic tumour cell lines. In this context, the enzyme functions to penetrate underlying basement membranes and allow the tumour cell to escape from the site of primary tumour formation and enter the circulation. After adhering to blood vessel walls, the tumour cells use these same metalloproteinases to pierce underlying basement membranes and penetrate other tissues, thereby leading to tumour metastasis. Inhibition of this process would prevent metastasis and improve the efficacy of current treatments with chemotherapeutics and/or radiation.

These inhibitors should also be useful for controlling periodontal diseases, such as gingivitis. Both collagenase and stromelysin activities have been isolated from fibroblasts derived from inflamed gingiva (Uitto *et al* (1981), *J. Periodontal Res.*, 16:417-424). Enzyme levels have been correlated to the severity of gum disease; C. M. Overall *et al* (1987), *J. Periodontal Res.*, 22:81-88.

Proteolytic processes have also been observed in the ulceration of the cornea following alkali burns (S. I. Brown *et al* (1969), *Arch. Ophthalmol.*, 81:370-373). Mercapto-containing peptides do inhibit the collagenase isolated from alkali-burned rabbit cornea (F. R. Burns *et al* (1989), *Invest. Ophthalmol*, 30:1569-1575). Treatment of alkali-burned eyes or eyes exhibiting corneal ulceration as a result of infection with inhibitors of these metalloendoproteinases in combination with sodium citrate or sodium ascorbate and/or antimicrobials may be effective in preventing developing corneal degradation.

Stromelysin has been implicated in the degradation of structural components of the glomerular basement membrane (GBM) of the kidney, the major function of which is to restrict passage of plasma proteins into the urine (W. H. Baricos *et al* (1989), Biochem. J., 254:609-612). Proteinuria, a result of glomerular disease, is excess
5 protein in the urine caused by increased permeability of the GBM to plasma proteins. The underlying causes of the increased GBM permeability are unknown, but proteinases including stromelysin may play an important role in glomerular diseases. Inhibition of this enzyme may alleviate the proteinuria associated with kidney malfunction.

It is suggested that inhibition of matrix metalloproteinase activity may prevent
10 the rupturing of atherosclerotic plaques leading to coronary thrombosis. The tearing or rupture of atherosclerotic plaques is the most common event initiating coronary thrombosis. Destabilisation and degradation of the connective tissue matrix surrounding these plaques by proteolytic enzymes or cytokines released by infiltrating inflammatory cells has been proposed as a cause of plaque fissuring. Such tearing of these plaques
15 can cause an acute thrombolytic event as blood rapidly flows out of the blood vessel. High levels of stromelysin RNA message have been found to be localised to individual cells in atherosclerotic plaques removed from heart transplant patients at the time of surgery (A. M. Henney *et al* (1991), Proc. Nat'l. Acad. Sci. USA, 88:8154-8158). Inhibition of stromelysin by these compounds may aid in preventing or delaying the
20 degradation of the connective tissue matrix that stabilises the atherosclerotic plaques, thereby preventing events leading to acute coronary thrombosis.

It has been recently shown in a model of congestive heart failure (CHF) in the pig, that during CHF there are marked changes in the morphological structure of the heart. Ventricular dilation and wall thinning caused by changes to the extracellular
25 matrix results in fewer collagen connections between cardiomyocytes and less total collagen. In such an instance a weaker force of contraction leads to an inefficient ventricular operation. It is believed that specific inhibitors of matrix metalloproteinases will play a key role in stabilising the extracellular matrix and therefore be important in the treatment and/or prevention of CHF.

It has recently been shown (WO 96/0240) that inhibitors of the matrix metalloproteinases, such as collagenase and stromelysin also inhibit the formation of human soluble CD23. CD23 is a 45kDa type II integral protein expressed on the surface of a variety of mature cells, including B and T lymphocytes, macrophages, NK cells, Langerhans cells, monocytes, eosinophils and platelets (Delespesse *et al* (1991), Adv. Immunology, 49:149; Grangette *et al* (1989), J., Immunol, 143:3580). Several activities have been ascribed to soluble CD23 in man, all of which involve IgE regulation. Particular activities include:

- i) antigen presentation
- ii) IgE mediated eosinophil cytotoxicity
- iii) B cell homing to lymph nodes and the spleen
- iv) downregulation of IgE synthesis

Thus, overall the excessive production of soluble CD23 has been implicated in the overproduction of IgE, the hallmark of allergic diseases such as extrinsic asthma, rhinitis, allergic conjunctivitis, eczema, atopic dermatitis and anaphylaxis (Sutton *et al* (1993), Nature, 366:421). Elevated levels of soluble CD23 have also been observed in the serum of patients with chronic B lymphocytic leukaemia (Safari *et al* (1988), Blood, 71:94), and in the synovial fluid of patients with rheumatoid arthritis (Chomarat *et al* (1993), Arthritis and Rheumatism, 36:234).

Recent reports suggest that new enzymes of the MMP family also mediate the shedding of adhesion molecules such as the selectins, such as L-selectin. These soluble adhesion molecules are implicated in a number of diseases including cancer, autoimmunity and in the inflammatory response. It has been proposed that once cleaved, the selectin bind to particular ligands and this accounts for their biological activity. Thus, drugs that interfere with or prevent binding of the ligands to the selectins will be useful medicaments for treating a variety of the diseases described above. Therefore, it is a yet further objective of the present invention to provide compounds which inhibit the shedding of certain adhesion molecules and thus provide the production of a

medicament for the treatment or prophylaxis of disorders such as cancer, autoimmune diseases or inflammatory diseases (such as inflammatory bowel disease and multiple sclerosis).

It is also believed that specific inhibitors of stromelysin and collagenase should be useful as birth control agents. There is evidence that expression of metalloproteinases, including stromelysin and collagenase, is observed in unfertilised eggs and zygotes and at further cleavage stages and increased at the blastocyst stage of fetal development and with endoderm differentiation (C. A. Brenner *et al* (1989), *Genes & Develop.*, 3:848-59). By analogy to tumour invasion, a blastocyst may express metalloproteinases in order to penetrate the extracellular matrix of the uterine wall during implantation. Inhibition of stromelysin and collagenase during these early development processes should presumably prevent normal embryonic development and/or implantation in the uterus. Such intervention would constitute a novel method of birth control. In addition there is evidence that collagenase is important in ovulation processes. In this example, a covering of collagen over the apical region of the follicle must be penetrated in order for the ovum to escape. Collagenase has been detected during this process and an inhibitor has been shown to be effective in preventing ovulation (J. F. Woessner *et al* (1989), *Steroids*, 54:491-499). There may also be a role for stromelysin activity during ovulation (C. K. L. Too *et al* (1984), *Endocrin.*, 115:1043-1050).

Collagenolytic and stromelysin activity have also been observed in dystrophic epidermolysis bullosa (A. Kronberger *et al* (1982), *J. Invest. Dermatol.*, 79:208-211; D. Sawamura *et al* (1991), *Biochem. Biophys. Res. Commun.*, 184:1003-8). Inhibition of metalloendoproteinases should limit the rapid destruction of connective components of the skin.

In addition to extracellular matrix comprising structural components, stromelysin can degrade other *in vivo* substrates including the inhibitors α_1 -proteinase inhibitor and may therefore influence the activities of other proteinases such as elastase (P. G. Winyard *et al* (1991), *FEBS Letts.*, 279,1:91-94). Inhibition of the matrix

metalloendoproteinases may potentiate the antiproteinase activity of these endogenous inhibitors.

From recent publications it is evident that several new enzymes of the MMP family have been identified, some of which may be important in disease. Collagenase 3, an enzyme unique to breast carcinoma cells may have utility in breast cancer (JMP Freije *et al* (1994), J. Biol. Chem., 269 (24): 16766-16773), whilst MT-MMPs, other members of the MMP family have been shown to be a key enzymes in the activation of gelatinase A (H Sato *et al* (1994), Nature, 370:61-65). Gelatinase A is an important enzyme in the growth and metastasis of tumours (such as defined above).

The degradation of b-Amyloid Precursor Protein (APP) has been shown to generate amyloid plaques, a major constituent of the senile plaques, found in patients with Alzheimers Disease (AD). Two recent publications have identified metalloproteinase enzymes that cleave APP to the amyloid plaque (CR Abraham *et al* (1994), Biochemistry, 33:192-199; G Huber *et al* (1994), Biochem. Biophys. Res. Comm., 201 (1):45-53).

As appreciated by those of skill in the art, the significant proportion of homology between these new enzymes and other MMPs leads to the possibility that a compound that inhibits one enzyme may to some degree inhibit these new enzymes. Therefore, inhibitors encompassed in this invention may be useful in the diseases in which these new enzymes are implicated.

It is, therefore, a further objective of this invention to provide compounds which, in addition to inhibiting TNF release, also inhibit the action of MMPs, and hence may be used in the treatment of patients who suffer from conditions mediated by TNF and/or MMPs.

It is therefore, a further objective of the present invention to provide compounds which inhibit the formation of human soluble CD23 for the production of a medicament for the treatment or prophylaxis of disorders such as allergy and autoimmune disease in which the overproduction of soluble CD23 is implicated, such as those described above.

SUMMARY OF THE INVENTION

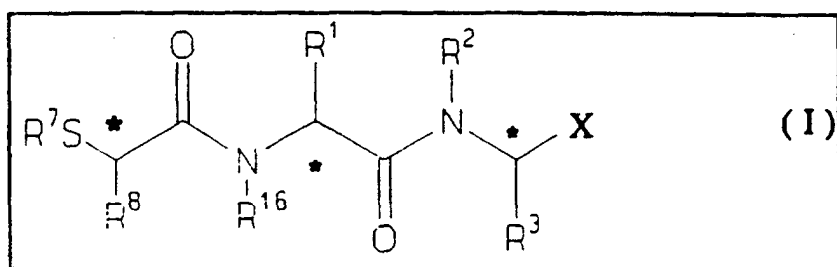
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The invention encompasses novel mercaptoalkylpeptidyl compounds of formula (I) which are useful inhibitors of matrix metalloproteinases and/or $\text{TNF}\alpha$ mediated diseases including degenerative diseases (such as defined above and in WO-A-9611209) and certain cancers.

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In a first aspect of the invention there is provided a compound of general formula (I):

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wherein:

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R^1 may be C_{1-7} alkyl, C_{2-6} alkenyl, C_{1-6} alkyl-aryl, aryl, C_{1-6} alkyl-heteroaryl, heteroaryl or C_{1-6} alkyl- AR^9 group where A is O, NR^9 or $S(O)_m$ where $m = 0-2$, and R^9 is H, C_{1-4} alkyl, aryl, heteroaryl, C_{1-4} alkyl-aryl or C_{1-4} alkyl-heteroaryl; if $A = NR^9$ the groups R^9 may be the same or different;

20

R^2 is hydrogen or a C_{1-6} alkyl group;

R^3 is a $[Alk]_nR^6$ group where Alk is a C_{1-6} alkyl or C_{2-6} alkenyl group and n is zero or 1;

25

X is heteroaryl or a group $CONR^4R^5$ where R^4 is hydrogen or an C_{1-6} alkyl, aryl, heteroaryl, C_{1-6} alkyl-heteroaryl, cyclo(C_{3-6})alkyl, C_{1-6} alkyl-cyclo(C_{3-6})alkyl, heterocyclo(C_{4-6})alkyl or C_{1-6} alkyl-heterocyclo(C_{4-6})alkyl group and R^5 is hydrogen or a C_{1-6} alkyl group; NR^4R^5 may also form a ring such as pyrrolidino, piperidino or morpholino;

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R^7 is hydrogen or the group $R^{10}CO$ where R^{10} is C_{1-4} alkyl, $(C_{1-4}$ alkyl)aryl, $(C_{1-4}$ alkyl)heteroaryl, cyclo(C_{3-6}) alkyl, cyclo(C_{3-6}) alkyl C_{1-4} alkyl, C_{2-6} alkenyl, C_{2-6} alkenylaryl, aryl or heteroaryl as defined above;

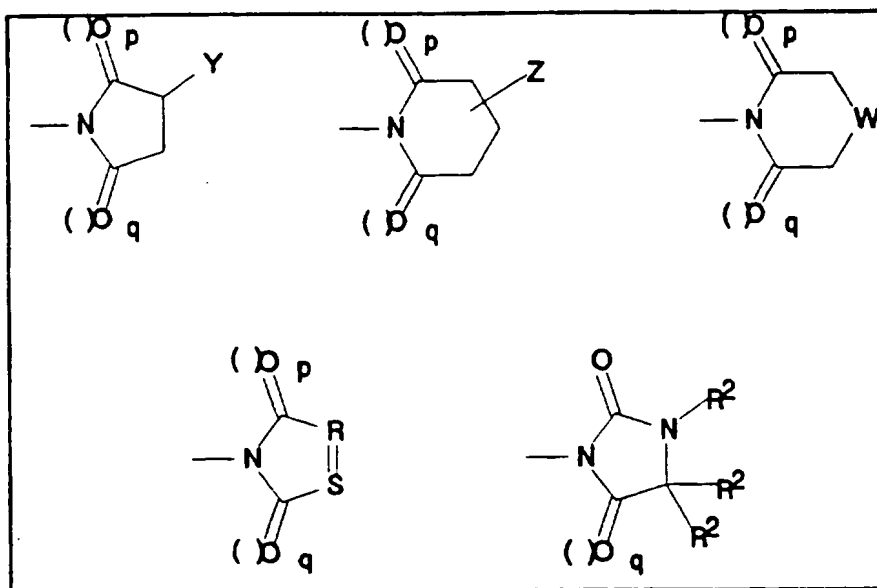
5 R^8 and R^{16} are the same or different and are each C_{1-4} alkyl R^{11} , R^{16} may also be H;

R^6 represents AR^9 or cyclo(C_{3-6})alkyl, cyclo(C_{3-6})alkenyl, C_{1-6} alkyl, C_{1-6} alkoxyaryl, benzyloxyaryl, aryl, heteroaryl, $(C_{1-3}$ alkyl)heteroaryl, $(C_{1-3}$ alkyl)aryl, C_{1-6} alkyl-COOR⁹, C_{1-6} alkyl-NHR¹⁰, CONHR¹⁰, NHCO₂R¹⁰, NHSO₂R¹⁰, NHCOR¹⁰, amidine or
 10 guanidine;

R^{11} is COR¹³ or NHCOR¹³ or the groups

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where p and q are each 0 or 1 and are the same or different but when $p=q=1$, Y cannot be H;

R and S are CH or N and are the same or different;

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W is O, $S(O)_m$ where $m=0,1$ or 2 , or NR^{12} ;

Y and Z are each H or C_{0-4} alkyl R^{14} wherein R^{14} is NHR^2 , $N(R^2)_2$ (where each R^2 may be the same or different), $COOR^2$, $CONHR^2$, $NHCO_2R^2$ (where R^2 is not H), $NHSO_2R^2$ (where R^2 is not H) or $NHCO_2R^2$; Z may be attached to any position on the ring;

R^{12} is hydrogen, C_{1-4} alkyl, COR^9 , CO_2R^9 (where R^9 is not H), $CONHR^9$, or SO_2R^9 (where R^9 is not H);

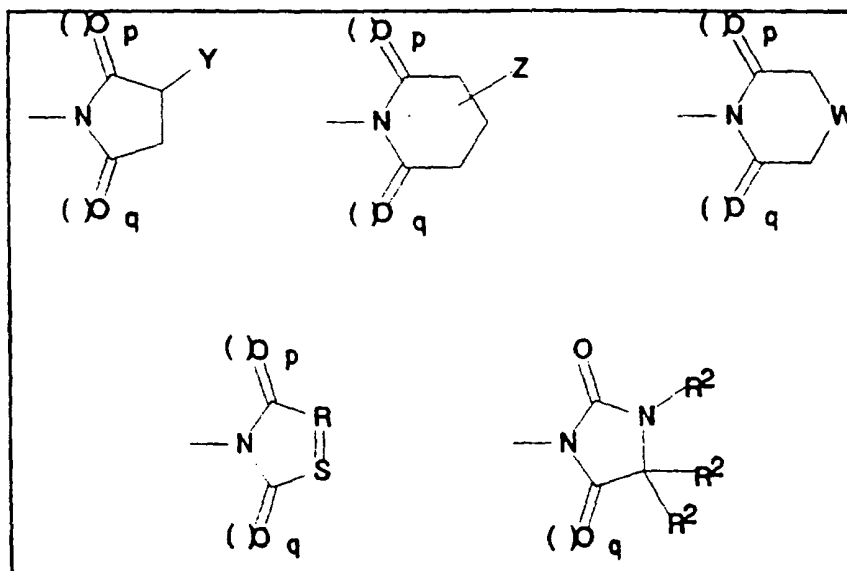
10 R^{13} is $(C_{1-4}$ alkyl) R^{15} ;

R^{15} is $N(R^9)_2$ (where each R^9 may be the same or different), CO_2R^9 , $CONHR^9$, $CON(R^9)_2$ (where each R^9 may be the same or different) or SO_2R^9 (where R^9 is not H), phthalimido or the groups

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30 and the salts, solvates and hydrates thereof.

Description of the Invention

It will be appreciated that the compounds according to the invention can contain one or more asymmetrically substituted carbon atoms, for example those marked with an asterisk in formula (1). The presence of one or more of these asymmetric centres
5 in a compound of formula (1) can give rise to stereoisomers, and in each case the invention is to be understood to extend to all such stereoisomers, including enantiomers and diastereomers, and mixtures including racemic mixtures thereof.

In the formulae herein, the ~ line is used at a potential asymmetric centre to represent the possibility of *R*- and *S*- configurations, the < line and the line to
10 represent a unique configuration at an asymmetric centre.

As used in this specification, alone or in combination, the term " C_{1-7} alkyl" refers to a straight or branched chain alkyl moiety having from one to six carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, *tert*-butyl, pentyl, hexyl, heptyl and the like. " C_{1-6} alkyl" means the same, up to 6 carbon atoms, e.g. hexyl.
15 " C_{1-4} alkyl" means the same, up to 4 carbon atoms, e.g. butyl or *tert*-butyl.

The term " C_{2-6} alkenyl" refers to a straight or branched chain alkyl moiety having two to six carbon atoms and having in addition one double bond, of either E or Z stereochemistry where applicable. This term would include for example, vinyl, 1-propenyl, 1- and 2- butenyl, 2- methyl-2-propenyl etc.

20 The term "cyclo(C_{3-6})alkyl" refers to a saturated alicyclic moiety having from three to six carbon atoms and includes for example cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

The term "cyclo(C_{3-6})alkenyl" refers to an alicyclic moiety having from three to six carbon atoms and having in addition one double bond. This term would include for
25 example cyclopentenyl or cyclohexenyl.

The term "heterocyclo(C_{4-6})alkyl" refers to a saturated heterocyclic moiety having from four to six carbon atoms and one or more heteroatoms selected from the group N, O, S and includes for example azetidiny, pyrrolidinyl, tetrahydrofuranyl, piperidinyl and the like.

The term "aryl" means an optionally substituted phenyl or naphthyl group with the substituent(s) being selected, for example, from halogen, trifluoromethyl, C₁₋₆ alkyl, alkoxy, phenyl and the like.

The term "halogen" means fluorine, chlorine, bromine or iodine.

5 The terms "protected amino" and "protected carboxy" mean amino and carboxy groups which are protected in a manner familiar to those skilled in the art. For example, an amino group can be protected by a benzyloxycarbonyl, *tert*-butoxycarbonyl, acetyl or like groups, or in the form of a phthalimido or like group. A carboxyl group can be protected in the form of a readily cleavable ester such as the methyl, ethyl,
10 benzyl or *tert*-butyl ester.

The term "alkoxy" refers to a straight chain or branched chain alkoxy group containing a maximum of six carbon atoms, such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, *tert*-butoxy and the like.

15 The term "heteroaryl" refers to aromatic ring systems of five to ten atoms or which at least one atom is selected from O, N and S and includes for example furanyl, thiophenyl, pyridyl, indolyl, quinolyl and the like.

Salts of compounds of formula (I) include pharmaceutically acceptable salts, for example acid addition salts derived from inorganic or organic acids, such as hydrochlorides, hydrobromides, p-toluenesulphonates, phosphates, sulphates,
20 perchlorates, acetates, trifluoroacetates, propionates, citrates, malonates, succinates, lactates, oxalates, tartrates and benzoates.

Salts may also be formed with bases. Such salts include salts derived from inorganic or organic bases, for example alkali metal salts such as magnesium or calcium salts, and organic amine salts such as morpholine, piperidine, dimethylamine or
25 diethylamine salts.

When the "protected carboxy" group in compounds of the invention is an esterified carboxyl group, it may be a metabolically labile ester of formula CO₂R¹⁷ where R¹⁷ may be an ethyl, benzyl, phenethyl, phenylpropyl, α - or β -naphthyl, 2,4-dimethylphenyl, 4-*tert*-butylphenyl, 2,2,2-trifluoroethyl, 1-(benzyloxy)benzyl, 1-

(benzyloxy)ethyl, 2-methyl-1-propionyloxypropyl, 2,4,6-trimethylbenzyloxymethyl or pivaloyloxymethyl group.

Compounds of the general formula (I) may be prepared by any suitable method known in the art and/or by the following processes, which itself forms part of the invention.

According to a second aspect of the invention, there is provided a process for preparing a compound of general formula (I) as defined above. It will be appreciated that where a particular stereoisomer of formula (I) is required, the synthetic processes described herein may be used with the appropriate homochiral starting material and/or isomers may be resolved from mixtures using conventional separation techniques (e.g. HPLC).

The compounds according to the invention may be prepared by the following process. In the description and formulae below the groups R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , A, B, C, R, S, W, X, Y and Z are as defined above, except where otherwise indicated. It will be appreciated that functional groups, such as amino, hydroxyl or carboxyl groups, present in the various compounds described below, and which it is desired to retain, may need to be in protected form before any reaction is initiated. In such instances, removal of the protecting group may be the final step in a particular reaction. Suitable protecting groups for such functionality will be apparent to those skilled in the art. For specific details see "Protective Groups in Organic Synthesis", Wiley Interscience, T W Greene, PGM Wutts.

A process for preparing compounds of general formula (I) comprises deprotecting (for example by hydrolysis) a compound of the formula $R^7S-CHR^8-CO-NR^{16}-CHR^1-CO-NR^2-CHR^3-X$ (II), wherein R^7 represents a suitable protecting group (eg *tert*-butyl, trityl, benzoyl or acetate).

It will be appreciated that where a particular stereoisomer of formula (I) is required, this may be obtained by conventional resolution techniques such as high performance liquid chromatography. Where desired, however, appropriate homochiral

starting materials may be used in the coupling reaction to yield a particular stereoisomer of formula (I). This is exemplified below.

Intermediates of general formula (II) may be prepared by coupling an acid of the formula R^7S-CHR^8-COOH (III), wherein R^7 and R^8 are as defined above, or an active derivative thereof, with an amine of the formula $R^{16}NH-CHR^1-CO-NR^2-CHR^3-X$ (IV).
5 Active derivatives of acids of formula (III) include for example acid anhydrides or acid halides, such as acid chlorides.

The coupling reaction may be performed using standard conditions for amination reactions of this type. Thus, the reaction may be achieved in a solvent, for example an
10 inert organic solvent such as an ether, e.g. a cyclic ether such as tetrahydrofuran, an amide e.g. a substituted amide such as dimethylformamide, or a halogenated hydrocarbon such as dichloromethane at a low temperature e.g. $-30^{\circ}C$ to ambient temperature, such as $-20^{\circ}C$ to $0^{\circ}C$, optionally in the presence of as base, e.g. an organic base such as an amine, e.g. triethylamine or a cyclic amine such as N-methylmorpholine. Where an acid of formula (III) is used, the reaction may additionally
15 be performed in the presence of a condensing agent, for example a diimide such as N,N'-dicyclohexylcarbodiimide, advantageously in the presence of a triazole such as 1-hydroxybenzotriazole. Alternatively, the acid may be reacted with a chloroformate for example ethylchloroformate, prior to reaction with the amine of formula (IV).

20 The amine of general formula (IV) may be prepared by coupling an acid of the formula $R^{16}NH-CHR^1-COOH$ (V), or an active derivative thereof, with an amine of the formula $R^2NH-CHX-R^3$ (VI) followed by removal of any protecting groups.

Active derivatives of acids of formula (V) include for example acid anhydrides or acid halides such as acid chlorides as outlined earlier.

25 Amino acids and their derivatives as depicted by general formulae (V) and (VI) can be obtained in chiral or racemic form. In the chiral form they provide asymmetric building blocks for the chiral synthesis of compounds of general formula (I). Many of these derivatives can be readily obtained from commercially available starting materials using methods known to those skilled in the art. (See "The Practice of Peptide
30 Synthesis" by M. Bodanszky et al, Springer Verlag, New York, 1984; WO92/21360).

Compounds of general formula (II) may be prepared by nucleophilic substitution of compounds of the formula $R^8R^{18}C-CO-NR^{16}-CHR^1-CO-NR^2-CHR^3-X$ (VII), wherein R^{18} represents a suitable leaving group (e.g. a halogen such as bromide, or an alkylsulphonate ester such as methanesulphonate) with a thiol of the formula R^7SH (VIII), using standard conditions known to those skilled in the art as exemplified in WO 90/05719.

Thiols of formula (VIII) may be obtained from commercially available starting materials using methods known to those skilled in the art. Many thiols of general formula (VIII) are also commercially available.

Compounds of formula (VII) may be prepared by coupling an acid of the formula $R^{18}R^3CH-COOH$ (IX), with an amine of formula (IV), wherein R^{18} and R^8 are as defined above (or suitably protected versions thereof) or an active derivative thereof, using similar coupling conditions to those described for the preparation of compounds of formula (II).

Carboxylic acids of the structures depicted in formulae (III) and (IX) can be obtained in chiral or racemic form. Many of these derivatives can be readily obtained from commercially available starting materials using methods known to those skilled in the art (see WO 90/05719).

Intermediates of formula (II) may be prepared by coupling an acid of the formula $R^7S-CHR^8-CO-NR^{16}-CHR^1-COOH$ (X), wherein R^1 , R^7 and R^8 are as defined above, or an active derivative thereof, with an amine of formula (VI) by the procedure described previously.

Acids of general formula (X) may in turn be prepared by coupling an acid of formula (III), or an active derivative thereof with an amine of formula (V), or a suitably protected derivative thereof, followed by removal of any protecting groups.

Active derivatives of acids of formula (X) include for example acid anhydrides or acid halides such as acid chlorides as outlined earlier.

Compounds of formula (I) may also be prepared by interconversion of other compounds of formula (I). Thus, for example, a compound of formula (I) wherein R^1 is a C_{1-6} alkyl group may be prepared by hydrogenation (using palladium on carbon in

suitable solvent, such as an alcohol - e.g. ethanol) of a compound of formula (I) wherein R^1 is a C_{2-6} alkenyl group. A further example would include a compound of formula (I) wherein R^7 is a group $R^{10}CO$ may be prepared by acylation (using a suitable acid chloride $R^{10}COCl$, in the presence of a base such as a triethylamine in a suitable solvent, such as a chlorinated solvent - eg dichloromethane) of a compound of formula (I) wherein R^7 is H.

Compounds where X is heteroaryl may be prepared according to the procedures described in PCT/GB96/01137.

Any mixtures of final products or intermediates obtained can be separated on the basis of the physico-chemical differences of the constituents, in known manner, into the pure final products or intermediates, for example by chromatography, distillation, fractional crystallization, or by formation of a salt if appropriate or possible under the circumstances.

The compounds according to the invention exhibit *in vitro* inhibiting activities with respect to stromelysin, collagenase and gelatinase. Compounds according to the invention also exhibit *in vitro* inhibition of $TNF\alpha$ release. The activity and selectivity of the compounds may be determined by use of the appropriate enzyme inhibition test, for example as described in Example A below and in WO-A-9611209. The same publication gives other tests (Examples B to G) appropriate for testing compounds of this invention.

This invention also relates to a method of treatment for patients (including man and/or mammalian animals raised in the dairy, meat or fur industries or as pets) suffering from disorders or diseases which can be attributed to matrix metalloproteinases and/or $TNF\alpha$ as previously described, and more specifically, a method of treatment involving the administration of the matrix metalloproteinase inhibitors of formula (I) as the active constituents.

Accordingly, the compounds of formula (I) can be used among other things in the treatment of osteoarthritis and rheumatoid arthritis, and in diseases and indications resulting from the over-expression of these matrix metalloproteinases such as found in certain metastatic tumour cell lines.

As mentioned above, compounds of formula (I) are useful in human or veterinary medicine since they are active as inhibitors of $\text{TNF}\alpha$ and MMPs. Accordingly in another aspect, this invention concerns:

5 a method of management (by which is meant treatment or prophylaxis) of disease or conditions mediated by $\text{TNF}\alpha$ and/or MMPs in mammals, in particular in humans, which method comprises administering to the mammal an effective, amount of a compound of formula (I) above, or a pharmaceutically acceptable salt thereof; and

a compound of formula (I) for use in human or veterinary medicine, particularly in the management (by which is meant treatment or prophylaxis) of diseases or
10 conditions mediated by $\text{TNF}\alpha$ and/or MMPs; and

the use of a compound of formula (I) in the preparation of an agent for the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by $\text{TNF}\alpha$ and/or MMPs.

The disease or conditions referred to above include inflammatory diseases,
15 autoimmune diseases cancer, cardiovascular diseases, diseases involving tissue breakdown such as rheumatoid arthritis, osteoarthritis, osteoporosis, neurodegeneration, Alzheimer's disease, atherosclerosis, congestive heart failure, stroke, vasculitis, Crohn's disease, ulcerative colitis, multiple sclerosis, periodontitis, gingivitis and those involving tissue breakdown such as bone resorption, hydroxy-haemorrhage, coagulation, acute
20 phase response, cachexia and anorexia, acute infections, HIV infections, fever, shock states, graft versus host reactions, dermatological conditions, surgical wound healing, psoriasis, atopic dermatitis, epidermolysis bullosa, tumour growth, angiogenesis and invasion by secondary metastases, ophthalmological disease, retinopathy, corneal ulceration, reperfusion injury, migraine, meningitis, asthma, rhinitis, allergic
25 conjunctivitis, eczema and anaphylaxis.

For the treatment of rheumatoid arthritis, osteoarthritis, and in diseases and indications resulting from the over-expression of matrix metalloendoproteinases such as found in certain metastatic tumour cell lines or other diseases mediated by the matrix metalloendoproteinases or increased $\text{TNF}\alpha$ production, the compounds of formula (I)
30 may be administered orally, topically, parenterally, by inhalation spray or rectally in

dosage unit formulations containing non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, cattle, sheep, dogs, cats etc, the compounds of the invention are effective in the treatment of humans.

The pharmaceutical composition containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the techniques described in the US Patents 4,256,108; 4,166,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

Formulations for oral use may also be presented as hard gelatin capsules where in the active ingredient is mixed with an inert solid diluent, for example calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active

ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as a polyoxyethylene with partial esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified, for example sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or

arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally- occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soya bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavouring and colouring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be in a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds of formula (I) may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc containing the compounds of Formula (I) are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

Dosage levels of the order of from about 0.05 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 2.5 mg to about 7 gms per patient per day). For example, inflammation may be effectively treated by the administration of from about 0.01 to 50 mg of the compound per kilogram of body weight per day (about 0.5 mg to about 3.5 gms per patient per day).

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may vary from about 5 to about 95 percent of the total composition. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy. The following non-limiting Examples are intended to illustrate the preparation of compounds of Formula (1), and as such are not intended to limit the invention as set forth in the claims.

In the Examples, the following abbreviations are used:

TNF _α	Tumour necrosis factor α
LPS	Lipopolysaccharide
ELISA	Enzyme linked immunosorbent assay
RT	Room Temperature
EDC	1-Ethyl-3-(Dimethylaminopropyl)carbodiimide hydrochloride
<u>Intermediate 1</u>	[(2 <i>R</i> -Bromo-5-phthalimido]pentanoyl-L-leucyl-L- <i>tert</i> -leucine N-methylamide

EDC (1.47 g, 7.71 mmol) was added to a solution of [(2*R*)-bromo-5-phthalimido]pentenoic acid (see WO-A-9611209, intermediate 117, 2.40 g, 7.35 mmol) and N-hydroxybenzotriazole (1.04 g, 7.71 mmol) in tetrahydrofuran (40 ml) at 3°C. L-Leucyl-L-*tert*-leucine N-methylamide (see WO-A-9611209, intermediate 116, 1.89 g, 7.35 mmol) was added, the mixture was allowed to warm slowly to RT and stirred overnight. The mixture was partitioned between ethyl acetate and water and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with 1N HCl, sat. sodium bicarbonate solution and brine, dried (MgSO₄) and evaporated *in vacuo* to give the title compound (3.93 g, 95 %) as a white foam which was used in the next step without further purification.

TLC R_f 0.25 (5% MeOH-CH₂Cl₂)

Intermediate 2 [(2 *S*) - T r i p h e n y l m e t h y l s u l f a n y l - 5 - phthalimido]pentanoyl-L-leucyl-L-*tert*-leucine N-methylamide

Potassium *tert*-butoxide (677 mg, 6.03 mmol) was added to a stirred solution of triphenylmethylmercaptan (1.66 g, 6.03 mmol) in dimethylformamide (70 ml) at 3°C. After stirring for 20 min intermediate 1 (3.25 g, 5.75 mmol) was added and the mixture was allowed to warm slowly to RT and stirred overnight. The mixture was then poured into water (200 ml) and the resulting precipitate, collected by filtration and dried *in vacuo* to give the title compound (3.12 g, 71 %) as a pale yellow solid.

TLC R_f 0.47 (5% MeOH-CH₂Cl₂)

Intermediate 3 [(2*S*)-Triphenylmethylsulfanyl-5-amino]pentanoyl-L-leucyl-L-*tert*-leucine N-methylamide

40 % aqueous methylamine solution (10 ml, 116 mmol) was added to a solution of intermediate 2 (1.13 g, 1.48 mmol) in methanol (20 ml) at RT. The resulting suspension was stirred at RT overnight. The solvent was evaporated *in vacuo*, the residue dissolved in ethyl acetate and washed with water and brine, dried (MgSO₄) and
5 evaporated *in vacuo* to give the title compound (850 mg, 91 %) as an orange foam.

TLC R_f 0.27 (5% MeOH-CH₂Cl₂)

10 Intermediate 4 [(2S)-Triphenylmethylsulfanyl-5-[(N,N-dimethylamino)acetyl]amino
pentanoyl-L-leucyl-L-*tert*-leucine N-methylamide

EDC (65 mg, 0.34 mmol) was added to a stirred solution of intermediate 3 (200 mg, 0.32 mmol), N,N-dimethylglycine (33 mg, 0.32 mmol) and N-hydroxybenzotriazole (46 mg, 0.34 mmol) in tetrahydrofuran (15 ml) at 3°C. The mixture was allowed to
15 warm slowly to RT and stirred overnight. The mixture was then partitioned between ethyl acetate and water and the aqueous phase extracted with ethyl acetate. The combined organic extracts were washed with sat. sodium bicarbonate solution and brine, dried (MgSO₄) and evaporated *in vacuo* to give the crude product as a colourless oil. Purification by flash column chromatography, eluting with 2-3 % methanol-
20 dichloromethane, provided the title compound (163 mg, 0.23 mmol, 71 %) as a white solid.

TLC R_f 0.36 (5% MeOH-CH₂Cl₂)

25 Similarly prepared was:

Intermediate 5 [(2S)-Triphenylmethylsulfanyl-5-[[N-methyl-N-(1,1-dimethylethoxy carbonyl]aminoacetyl] aminopentanoyl-L-leucyl-L-*tert*-leucine
N-methylamide

From intermediate 3 (250 mg, 0.40 mmol), N-(1,1-dimethylethoxycarbonyl)sarcosine (76 mg, 0.40 mmol), EDC (77 mg, 0.40 mmol) and N-hydroxybenzotriazole (54 mg, 0.40 mmol). The crude product was obtained as a colourless oil. Purification by flash column chromatography, eluting with 2-3 %
5 methanol-dichloromethane, provided the title compound (260 mg, 0.32 mmol, 81 %) as a white solid.

TLC R_f 0.47 (5% MeOH-CH₂Cl₂)

10 Intermediate 6 *Tert*-Butyl 5-bromobutyrate

Boron trifluoride etherate (2 ml) was added to a mixture of 5-bromobutyric acid (8.3 g, 50 mmol) and *tert*-butyl 2,2,2-trichloroacetimidate (10.5g, 50mmol) in dichloromethane (15 ml) and hexane (50 ml) at RT. The mixture was stirred at RT for
15 a further 18 h, then quenched by addition of sodium bicarbonate (5 g). The mixture was then filtered through Celite and the filtrate evaporated *in vacuo* to provide the title compound (7.2 g, 64 %) as a colourless oil.

TLC R_f 0.72 (25 % Ether-Hexane).

20

Intermediate 7 1,5,5-Trimethyl-3-(3-*tert*-butoxycarbonylpropyl)hydantoin

Sodium hydride (60%, 1.3 g, 32 mmol) was added to a solution of 1,5,5-trimethylhydantoin (4.0 g, 28.2 mmol) in dimethylformamide (10ml) at 0°C and
25 the mixture was stirred under nitrogen for 30 min. A solution of intermediate 7 (7.1 g) was then added, and the resulting mixture was stirred overnight at RT, then poured into water (100 ml) and extracted with *tert*-butyl methyl ether (100ml). The organic phase was washed with water and brine, dried (MgSO₄) and evaporated *in vacuo* to furnish the title compound (3.7 g, 46%) as a colourless oil.

30

TLC R_f 0.25 (2:1 Ether-Hexane)

Intermediate 8 1,5,5-Trimethyl-3-(3carboxypropyl)hydantoin

5 Trifluoroacetic acid (10 ml) was added to a solution of intermediate 7 (3.6 g) in dichloromethane (10 ml) at RT and the solution was stirred for 18 h. The resulting solution was evaporated *in vacuo* and the residual trifluoroacetic acid was azeotroped with toluene (3 x 50 ml) to give provide the title compound as colourless viscous oil, which was used directly in the next step.

10

TLC R_f 0.45 (Ether).

Intermediate 9 1 , 5 , 5 - T r i m e t h y l - 3 - (3 - b r o m o - 3
carboxypropyl)hydantion

15

A solution of the crude intermediate 8 was stirred in dichloroethane (10 ml) containing thionyl chloride (1.1 ml) for 3 h, then heated to 80°C for 30 min. Phosphorus trichloride (0.11 ml) was added, followed by bromine (2.5 g) and the mixture heated at 80°C for 3 h. The solution was then cooled, water (10 ml) was
20 cautiously added and the biphasic mixture was stirred at 50°C for 72 h. Further water (100ml) was then added and the mixture basified with sodium bicarbonate, then washed with ether. The aqueous phase was acidified with 2M hydrochloric acid to pH 2 and the mixture extracted with dichloromethane. The combined extracts were dried (MgSO₄) and evaporated *in vacuo* to provide the title compound (3.5g, 87%), as a colourless
25 viscous oil.

TLC R_f 0.45 (Ether).

Intermediate 10 1,5,5-Trimethyl-3-(3-acetylthio-3-carboxypropyl)hydantoin

30

A solution of intermediate 10 (3.5 g) in methanol (20 ml) was treated with potassium thioacetate (1.56 g) at RT. The mixture was then stirred for 18 h, evaporated *in vacuo* and the residue partitioned between 0.5M hydrochloric acid and dichloromethane. The organic phase was washed with water and brine, dried (MgSO₄) and evaporated *in vacuo* to give the title compound (3.0 g, 88 %) as an orange solid.

TLC R_f 0.52 (Ether).

The following were prepared, according to the procedure outlined for L-Leucyl-L-*tert*-leucine N-methylamide (intermediate 116 in WO-A-9611209)

Intermediate 11 L-(S-Methyl)cysteiny-L-Leucyl-L-*tert*-leucine N-methylamide

Intermediate 12 L-Norvalinyl-L-*tert*-leucine N-methylamide

15

Example 1 [(2*S*)-Sulfanyl-5-[(N,N-dimethylamino)acetyl]aminopentanoyl-L-leucyl-L-*tert*-leucine N-methylamide

Intermediate 4 (150 mg, 0.21 mmol) was dissolved in a mixture (10 ml) of trifluoroacetic acid (90 %), thioanisole (5 %), triisopropylsilane (2.5 %) and water (2.5 %) and the solution was stirred at RT overnight. The volatiles were evaporated *in vacuo* to give the crude product as a yellow solid. Purification by flash column chromatography on silica, eluting with 2-3 % methanol-dichloromethane, provided the title compound (59 mg, 0.12 mmol, 59 %) as a white solid.

25

TLC R_f 0.30 (5% MeOH-CH₂Cl₂)

Similarly prepared was:

Example 2 [(2*S*)-Sulfanyl-5-[(N-methylamino)acetyl]aminopentanoyl]-L-leucyl-L-*tert*-leucine N-methylamide

From intermediate 5 (220 mg, 0.27 mmol). The crude product was obtained as a yellow solid. Purification by flash column chromatography, eluting with 2-3 % methanol-dichloromethane, provided the title compound (92 mg, 0.16 mmol, 59 %) as a white solid.

TLC R_f 0.21 (5% MeOH-CH₂Cl₂)

10

Example 3 N-[2-(Acetylthio)-4-(1,5,5-trimethylhydantoinyl)butanoyl]-L-Leucyl-L-*tert*-leucine N-methylamide

A solution of L-leucyl-L-*tert*-leucine N-methylamide (0.4 g) and intermediate 10 (0.4 g) in dichloromethane (20ml) was treated with EDC (0.3 g) and the mixture stirred for 18 h at RT. The solution was washed with 0.5M hydrochloric acid and sodium bicarbonate, dried (MgSO₄) and evaporated *in vacuo* to provide the title compound (65%) as a beige foam.

20 TLC R_f 0.42 (10% MeOH-CH₂Cl₂)

Example 4 N-[2-(Acetylthio)-4-(1,5,5-trimethylhydantoinyl)butanoyl]-L-(*S*-methyl)cysteinyl-L-*tert*-leucine N-methylamide

25 From intermediate 10 and intermediate 11, as a beige foam (73%).

TLC R_f 0.37 (10% MeOH-CH₂Cl₂)

Example 5 N-[2-(Acetylthio)-4-(1,5,5-trimethylhydantoinyl)butanoyl]-L-norvalinyl-L-*tert*-leucine N-methylamide

From intermediate 10 and intermediate 12, as a beige foam (68%).

5

TLC R_f 0.35 (10% MeOH-CH₂Cl₂)

Example 6 N-[2-Sulphanyl-4-(1,5,5-Trimethylhydantoinyl)butanoyl]-L-~~Leuyl~~
L-*tert*-leucine N-methylamide

10

A solution of example 3 (0.4 g) in methanol (10 ml) was treated with ammonium hydroxide (SG 0.88, 1ml) at RT for 3 h. The mixture was evaporated *in vacuo*, partitioned between dichlorormethane and water, dried (MgSO₄) and evaporated *in vacuo* to provide the crude product as a beige solid. The residue was purified by flash column chromatography on silica, eluting with 5% methanol in dichlorormethane to provide the title compound (0.35 g, 83%), as a colourless solid.

15

TLC R_f 0.35 (10% MeOH-CH₂Cl₂)

20 Example 7 N-[2-Sulphanyl-4-(1,5,5-trimethylhydantoinyl)butanoyl]-L-(S-methyl)cysteinyl-L-*tert*-leucine N-methylamide

From example 5, as a colourless solid (85%)

25 Example 8 N-[2-Sulphanyl-4-(1,5,5,trimethylhydantoinyl)butanoyl]-L-norvalinyl-L-*tert*-leucine N-methylamide

From example 6, as a colourless solid (88%)

30 Example A

Collagenase inhibition activity

The potency of compounds of general formula (I) to act as inhibitors of collagenase was determined by the procedure of Cawston and Barrett, (Anal. Biochem., 99:340-345, 1979) whereby a 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with collagen and collagenase (buffered with 50 mM Tris, pH 7.6 containing 5 mM CaCl₂, 0.05% Brij 35, 60 mM NaCl and 0.02% NaN₃). The collagen was acetylated ³H or ¹⁴C-collagen prepared by the method of Cawston and Murphy (Methods in Enzymology, 80:711, 1981). The choice of radiolabel did not alter the ability of collagenase to degrade the collagen substrate. The samples were centrifuged to sediment undigested collagen and an aliquot of the radioactive supernatant removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1mM inhibitor, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the collagenase (IC₅₀).

Example B

Stromelysin inhibition activity

The potency of compounds of general formula (I) to act as inhibitors of stromelysin was determined using the procedure of Nagase *et al* (Methods in Enzymology Vol 254, 1994), whereby a 0.1 mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with stromelysin and ³H transferrin (buffered with 50 mM Tris, pH 7.6 containing 10 mM CaCl₂, 150M NaCl, 0.05% Brij, 35, and 0.02% NaN₃). The transferrin was carboxymethylated with ³H iodoacetic acid. The stromelysin activity in the presence of 1 mM, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the stromelysin (IC₅₀)

Example C

Gelatinase inhibition activity

The potency of the compounds of general formula (I) to act as inhibitors of gelatinase was determined using the procedure of Harris & Krane (Biochem Biophys. Acta, 258:566 - 576, 1972), whereby a 1 mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with gelatinase and heat denatured ³H or ¹⁴C-acetylated collagen (buffered with 50 mM Tris, pH 7.6 containing 5 mM CaCl₂, 0.05% Brij 35 and 0.02% NaN₃). The ³H or ¹⁴C gelatin was prepared by denaturing ³H or ¹⁴C-collagen produced according to the method of Cawston and Murphy (Methods in Enzymology, 80:711, 1981) by incubation at 60°C for 30 minutes. Undigested gelatin was precipitated by addition of trichloroacetic acid and centrifugation. The gelatinase activity in the presence of 1 mM, or dilution thereof, was compared to the activity in a control devoid of inhibitor and results reported as that inhibitor concentration effecting 50% inhibition of the gelatinase (IC₅₀).

15 Example D

MMP Inhibition Activity-Fluorimetric Assay

The potency of compounds of general formula (I) to act as inhibitors of collagenase-1(MMP-1), collagenase-2 (MMP-8), gelatinase-A (MMP-2), gelatinase-B (MMP-9) and stromelysin-1(MMP-3) was determined using the following procedure:

Inhibitors are dissolved in dimethylsulphoxide containing 0.02% b-mercaptoethanol and serial dilutions are prepared. Activated enzyme is incubated in assay buffer containing 50mM Tris, pH 7.4, 5mM CaCl₂, 0.002% NaN₃ and Brij 35 in the presence and absence of inhibitor. Samples are preincubated at 37°C for 15 minutes before the addition of the fluorimetric substrate (Mca-Pro-Leu-Dpa-Ala-Arg-NH₂) to a final concentration of 10mM. The assay is incubated for 90 minutes at 37°C and then read in a Fluoroscan II at I_{ex} (355nm) and I_{em} (460nm).

The enzyme activity was compared to activity in a control devoid of inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the stromelysin (IC₅₀).

Example EInhibition of TNF α production

The potency of the compounds of general formula (I) to act as inhibitors of the production of TNF α was determined using the following procedure. A 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37° C in an atmosphere of 5% CO₂ with THP-1 cells (human monocytes) suspended in RPMI 1640 medium and 20 μ M β -mercaptoethanol at a cell density of 1 x 10⁶/ml and stimulated with 5 μ g/ml final concentration of LPS. After 18 hours the supernatant is assayed for the levels of TNF α using a commercially available ELISA kit (R & D Systems).

The activity in the presence of 0.1mM inhibitor or dilutions thereof was compared to activity in a control devoid of inhibitor and results reported as that inhibitor concentration effecting 50% inhibition of the production of TNF α .

15 Example F

Adjuvant arthritic rat model

Compounds of general formula (I) were evaluated in an adjuvant arthritis model in the rat based on the methods employed by B.B. Newbould (1963), Br.J.Pharmacol, 21, 127-136 and C.M. Pearson and F.D. Wood (1959), Arthritis Rheum, 2, 440-459. Briefly male Wistar rats (180-200g) were injected at the base of the tail with Freund's adjuvant. Twelve days later the responding animals were randomised into experimental groups. Compounds of general formula (I) were dosed either orally as a suspension in 1% methyl cellulose or intraperitoneally in 0.2% carboxymethylcellulose from day 12 to the end of the experiment on day 22. Hind paw volumes were measured every two days from day 12 onwards and X-rays were taken of the hind feet on completion of the experiment. Results were expressed as the percent increase of foot volume over day 12 values.

30 Example G

Mouse ovarian carcinoma xenograft model

Compounds of general formula (I) were evaluated in an ovarian carcinoma xenograft model of cancer, based on that described by B. Davies *et al* (1993), Cancer Research, 53, 2087-2091. This model, in brief, consists of inoculating female nu/nu mice with 1×10^9 OVCAR3-icr cells into the peritoneal cavity. Compounds of general formula (I) are administered by the oral route as a suspension in 1% methyl cellulose or intraperitoneally as a suspension in phosphate buffered saline in 0.01% Tween-20. At the conclusion of the experiment (4-5 weeks) the number of peritoneal cells are counted and any solid tumour deposits weighed. In some experiments tumour development is monitored by measurement of tumour specific antigens.

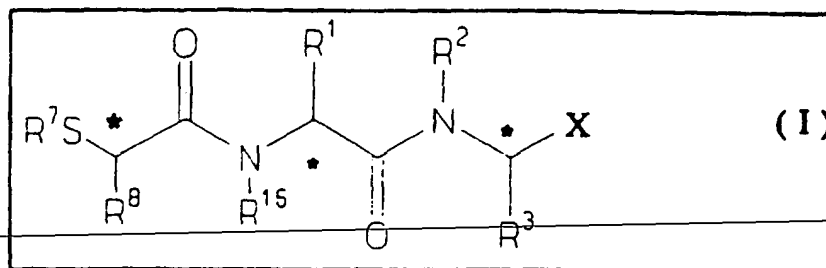
Example H

Rat mammary carcinoma model

Compounds of general formula (I) were evaluated in a HOSP.1 rat mammary carcinoma model of cancer (S.Eccles *et al* (1995), Cancer Research, in press). This model consists of the intravenous inoculation of female CBH/cbi rats with 2×10^4 tumour cells into the jugular vein. Compounds of general formula (I) are administered by the oral route as a suspension in 1% methyl cellulose or intraperitoneally as a suspension in phosphate buffered saline in 0.01% Tween-20. At the conclusion of the experiment (4-5 weeks) the animals were killed, the lungs were removed and individual tumours counted after 20 hours fixation in Methacarn.

CLAIMS

1. Compounds of general formula (I):



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wherein:

15 R^1 is C_{1-7} alkyl, C_{2-6} alkenyl, C_{1-6} alkyl-aryl, aryl, C_{1-6} alkyl-heteroaryl, heteroaryl or C_{1-6} alkyl- AR^9 group where A is O, NR^9 or $S(O)_m$ where $m = 0-2$, and R^9 is H, C_{1-4} alkyl, aryl, heteroaryl, C_{1-4} alkyl-aryl or C_{1-4} alkyl-heteroaryl; if $A = NR^9$ the groups R^9 may be the same or different.

R^2 is hydrogen or a C_{1-6} alkyl group;

20

R^3 is a $[Alk]_n R^6$ group where Alk is a C_{1-6} alkyl or C_{2-6} alkenyl group and n is zero or 1;

25 X is heteroaryl or a group $CONR^4R^5$ where R^4 is hydrogen or an C_{1-6} alkyl, aryl, heteroaryl, C_{1-6} alkyl-heteroaryl, cyclo(C_{3-6})alkyl, C_{1-6} alkyl-cyclo(C_{3-6})alkyl, heterocyclo(C_{4-6})alkyl or C_{1-6} alkyl-heterocyclo(C_{4-6})alkyl group and R^5 is hydrogen or C_{1-6} alkyl; NR^4R^5 may also form a ring such as pyrrolidino, piperidino or morpholino;

R^7 is hydrogen or the group $R^{10}CO$ where R^{10} is a group C_{1-4} alkyl, (C_{1-4} alkyl)aryl,

(C₁₋₆alkyl)heteroaryl, cyclo(C₃₋₆)alkyl, cyclo(C₃₋₆)alkyl-C₁₋₄ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkenylaryl, or aryl or heteroaryl as defined above;

R⁸ and R¹⁶ are the same or different and are each C₁₋₄ alkyl R¹¹, R¹⁶ may also be H;

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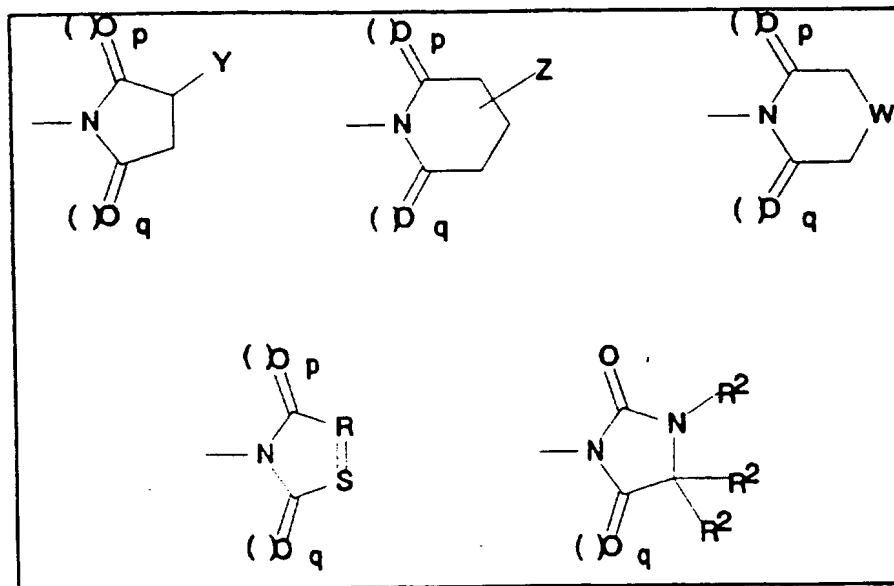
R⁶ represents AR⁹ or cyclo(C₃₋₆)alkyl, cyclo(C₃₋₆)alkenyl, C₁₋₆ alkyl, C₁₋₆ alkoxyaryl, benzyloxyaryl, aryl, heteroaryl, (C₁₋₃ alkyl)heteroaryl, (C₁₋₃ alkyl)aryl, C₁₋₆ alkyl-COOR⁹, C₁₋₆ alkyl-NHR¹⁰, CONHR¹⁰, NHCO₂R¹⁰, NHSO₂R¹⁰, NHCOR¹⁰, amidine or guanidine;

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R¹¹ is COR¹³, NHCOR¹³ or any of the groups

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25 where p and q are each 0 or 1 and are the same or different but when p=q=1, Y cannot be H;

R and S are each CH or N and are the same or different;

30 W is O, S(O)_m where m=0,1 or 2 or NR¹²;

Y and Z are each H or C_{0-4} alkyl R^{14} wherein R^{14} is NHR^2 , $N(R^2)_2$ (where each R^2 may be the same or different), $COOR^2$, $CONHR^2$, $NHCO_2R^2$ (where R^2 is not H), $NHSO_2R^2$ (where R^2 is not H) or $NHCOR^2$; Z may be attached to any position on the ring;

- 5 R^{12} is hydrogen, C_{1-4} alkyl, COR^9 , CO_2R^9 (where R^9 is not H), $CONHR^9$, or SO_2R^9 (where R^9 is not H);

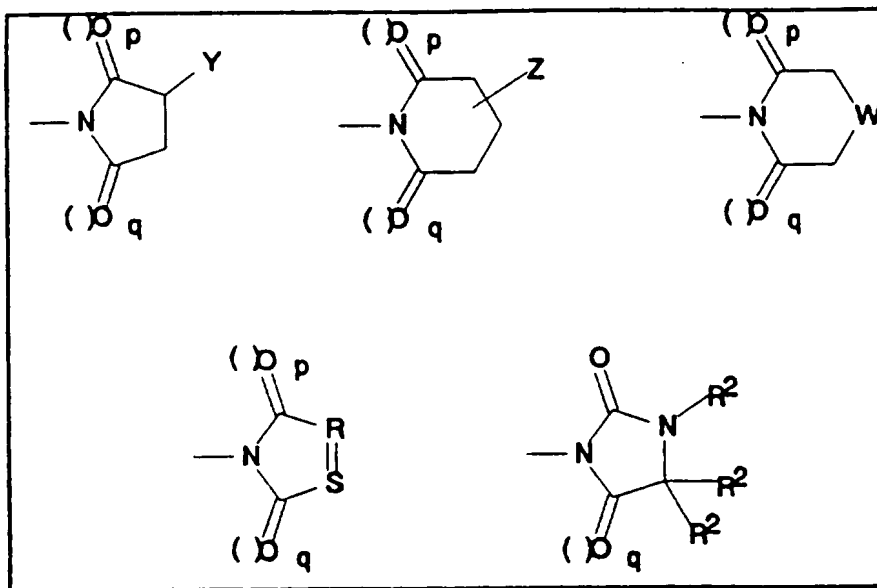
R^{13} is $(C_{1-4}$ alkyl) R^{15} ;

- 10 R^{15} is $N(R^9)_2$ (where each R^9 may be the same or different), CO_2R^9 , $CONHR^9$, $CON(R^9)_2$ (where each R^9 may be the same or different) or SO_2R^9 (where R^9 is not H), phthalimido or the groups

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and the salts, solvates and hydrates thereof.

2. A compound of claim 1, wherein X is $CONR^4R^5$; R^4 is H, alkyl or aryl; R^6 is not amidine or guanidine; R^{11} is not $NHCOR^{13}$ or the last of the given groups; R^{15} is not $N(R^2)_2$ or the last of the given groups; and R^{16} is H.
- 30

3. A compound of claim 1 selected from
[(2*S*)-Sulfanyl-5-[(N,N-dimethylamino)acetyl]aminopentanoyl-L-leucyl-L-*tert*-leucine]N-methylamide
[(2*S*)-Sulfanyl-5-[(N-methylamino)acetyl]aminopentanoyl-L-leucyl-L-*tert*-leucine N-methylamide.
- 5
4. A compound of claim 1 selected from
[(2*S*)-Acetylthio-4(1,5,5-trimethylhydantoinyl)butanoyl]-L-Leucyl-L-*tert*-leucine N-methylamide
[(2*S*)-Acetylthio-4(1,5,5-trimethylhydantoinyl)butanoyl]-L-(*S*-methyl)cysteinyl-L-*tert*-leucine N-methylamide
- 10
- [(2*S*)-Acetylthio-4(1,5,5-trimethylhydantoinyl)butanoyl]-L-norvalinyl-L-*tert*-leucine N-methylamide
N-[2-Sulfanyl-4-(1,5,5-trimethylhydantoinyl)butanoyl]-L-leucyl-L-*tert*-leucine N-methylamide
- 15
- N-[2-Sulfanyl-4-(1,5,5-trimethylhydantoinyl)butanoyl]-L-(*S*-methyl)cysteinyl-L-*tert*-leucine N-methylamide
N-[2-Sulfanyl-4-(1,5,5-trimethylhydantoinyl)butanoyl]-L-norvalinyl-L-*tert*-leucine N-methylamide
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5. A compound of any preceding claim in the form of a single enantiomer or diastereomer, or a mixture of such isomers.
6. A pharmaceutical composition for use in therapy, comprising a compound of any preceding claim, and a pharmaceutically-acceptable diluent or carrier.
7. Use of a compound of any of claims 1 to 5, for the manufacture of a human or veterinary medicament for the treatment or prevention of a condition associated with matrix metalloproteinases or that is mediated by TNF α or L-selectin sheddase.
- 25
8. Use according to claim 7, wherein the condition is selected from cancer, inflammation and inflammatory diseases, tissue degeneration, periodontal disease, ophthalmological disease, dermatological disorders, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute

infection, HIV infection, shock states, graft versus host reactions, autoimmune disease, reperfusion injury, meningitis and migraine.

- 5 9. Use according to claim 7, wherein the condition is selected from tumour growth, angiogenesis, tumour invasion and spread, metastases, malignant ascites and malignant pleural effusion.
10. Use according to claim 7, wherein the condition is selected from rheumatoid arthritis, osteoarthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's atherosclerosis, stroke, vasculitis, Crohn's disease and ulcerative colitis.
- 10 11. Use according to claim 7, wherein the condition is selected from corneal ulceration, retinopathy or surgical wound healing.
12. Use according to claim 7, wherein the condition is selected from psoriasis, atopic dermatitis, chronic ulcers epidermolysis bullosa.
13. Use according to claim 7, wherein the condition is selected from periodontitis
15 and gingivitis.
14. Use according to claim 7, wherein the condition is selected from rhinitis, allergic conjunctivitis, eczema and anaphalaxis.
15. Use according to claim 7, wherein the condition is selected from ristonosis, congestive heart failure, endometriosis, atherosclerosis and endosclerosis.

INTERNATIONAL SEARCH REPORT

Application No
PCT/GB 96/02438

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/03 C07K5/033 A61K38/07

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 95 06031 A (IMMUNEX CORP) 2 March 1995 see claims 1,18; examples ---	1,6-15
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

20 February 1997

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

Application No
PCT/GB 96/02438

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>PEPT.: CHEM. BIOL., PROC. AM. PEPT. SYMP., 12TH (1992), MEETING DATE 1991, 791-2. EDITOR(S): SMITH, JOHN A.; RIVIER, JEAN E. PUBLISHER: ESCOM, LEIDEN, NETH. CODEN: 57XGA9, 1992, XP002007758 ALMQUIST, RONALD G. ET AL: "Development of peptidomimetic inhibitors of a newly isolated atrial peptide-degrading enzyme" table 1, compound 9 see examples</p> <p style="text-align: center;">---</p>	1,7
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A	<p>BIOCHEM. BIOPHYS. RES. COMMUN. (1981), 101(4), 1251-8 CODEN: BBRC9; ISSN: 0006-291X, 1981, XP000575861 GRAY, ROBERT D. ET AL: "Metal binding peptide inhibitors of vertebrate collagenase" see page 1255, paragraph 2 - page 1257, paragraph 2; table I</p> <p style="text-align: center;">---</p>	1,7
E	<p>WO 96 35711 A (CHIROSCIENCE LTD ; BAXTER ANDREW DOUGLAS (GB); MONTANA JOHN (GB); O) 14 November 1996 see claims; examples</p> <p style="text-align: center;">---</p>	1-15
E	<p>WO 96 35712 A (CHIROSCIENCE LTD) 14 November 1996 see claims; examples</p> <p style="text-align: center;">-----</p>	1-15

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